

Improving bone properties and fracture susceptibility: experimental models of genetic manipulation, pharmacologic intervention, and cellular perturbation reveal new approaches for improving bone health
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Bone, a crucial support structure in the human body, is often taken for granted for its lightweight properties and unparalleled strength. Skeletal fracture is a major clinical condition affecting millions of Americans, which results from abnormal aging, hormonal imbalance, genetic conditions, and lifestyle choices (e.g., exercise). Because fractures are caused by a number of different factors, reducing fracture incidence requires a multifactorial approach to unraveling the underlying biology of bone metabolism, in order to discover new ways to improve bone properties and prevent fractures. We have taken such an approach by conducting (1) genetic manipulation experiments in mice, where genes predicted to be involved in bone mass regulation were mutated; (2) pharmacologic experiments to quantify the dose-response effect of an agent that inhibits bone loss, and (3) cell culture experiments, aimed at revealing molecular pathways activated by mechanical stimulation.

METHODS: Mice with mutations in two genes, likely to regulate bone mass (SOST, DKK1) were generated and subjected to *in vivo* dual energy x-ray absorptiometry (DEXA) scans at 6-wk old. Whole body scans were analyzed for bone mineral density (BMD) using Lunar Piximus II v2.10 software. Mice (6-wk) were also dosed (0, 1, 10, 100, or 1000 mg/kg) with daily alendronate HCl, a bisphosphonate that inhibits osteoclast activity. Six wks later, the mice were sacrificed, and the femurs were dissected and sectioned for histological analysis of bone formation parameters, including mineralizing surface (MS/BS), mineral apposition rate (MAR), and bone formation rate (BFR/BS). To understand the cellular signaling events in response to mechanical loading, bone marrow mesenchymal stem cells (MSCs) were treated with 10, 20, 30, or 40 μ M PF7408671, an S6 kinase inhibitor. Cells then were subject to 100 cycles of biaxial mechanical strain (2%, 10 cycles/min). Protein lysates were separated by electrophoresis and probed for phosphorylation of Rictor and Akt by Western blot.

RESULTS: Mice harboring mutations in either the SOST gene or the DKK1 gene exhibited significantly increased BMD compared to wild-type control mice, though the SOST mutation had a stronger effect on BMD than DKK1. Mice with compound mutations (SOST and DKK1 mutations) had significantly greater BMD than mice with either single mutation, suggesting that inhibition of SOST and DKK1 might be an effective means to increase bone mass in patients susceptible to fracture. Mice treated with high-dose alendronate (100 or 1,000 mg/kg) exhibited significant decreases in bone formation parameters (MS/BS, MAR, and BFR/BS) compared to untreated (0 mg) mice, suggesting that while this compound might be beneficial for inhibiting bone loss, it also inhibits bone formation. The signaling hub, mTORC2, is a critical regulator of mechanical force in MSC progenitors. Our data demonstrate that S6 kinase is an upstream activator of mTORC2 in response to mechanical strain.

CONCLUSION: Our experiments suggest that genetic manipulation of mice reveal viable protein targets (e.g., SOST, DKK1) that could ultimately be manipulated pharmacologically to improve bone mass. We also found that an FDA-approved class of drugs inhibits bone formation even at very low doses, suggesting that additional pro-anabolic compounds might benefit patients taking bisphosphonates. On a cell signaling level, we found that the mTORC2 pathway shows considerable promise for pharmacologic manipulation to simulate the effects of exercise. Taken together, these experiments highlight the utility of a broad approach to solving bone metabolism challenges that can affect fracture susceptibility.

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